MONOCHROMATIC ULTRA-VIOLET RADIATION AS AN ACTIVATING AGENT FOR THE EGGS OF ARBACIA PUNCTULATA

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It has been known since 1914 that *Arbacia* eggs can be activated by the radiation from a quartz mercury vapor lamp. Loeb (1914) reported membrane elevation in unfertilized sea-urchin eggs. He obtained cleavage in these same eggs by a subsequent treatment with hypertonic salt solution. The occasional production of parthenogenesis by ultra-violet radiation alone has been reported by Lillie and Baskervill (1922), Tschahotine (1921) and others.

We observed during the summer of 1936 that the radiation from a very intense mercury vapor lamp would activate readily the unfertilized eggs of *Arbacia punctulata*. During the summer of 1937 we have been able to obtain activation in *Arbacia* eggs with monochromatic radiation and found that certain wave-lengths in the ultra-violet region are highly effective in producing parthenogenesis.

Experimental Technique

Experiment with Polychromatic Radiation

An intense beam of radiation from a vertical water-cooled, high pressure quartz capillary mercury vapor lamp (Daniels and Heidt, 1932; Hollaender and Stauffer, 1933) was made to cover by means of a right angle quartz reflecting prism and a quartz condensing lens a horizontal area of 2 by 18 mm. *Arbacia* eggs freshly removed from the ovaries and washed were covered to the height of about 3 mm. with sea water in Syracuse watchglasses, and moved through the beam in such a manner that the dishes were exposed for about 1 to 10 seconds, each egg thus receiving only a fraction of a second of exposure. The eggs in dishes which had received the appropriate quantities of radiation (3 to 5 seconds/dish) showed up to 90 per cent cleavage 3½ to 5 hours after exposure. The activation was not interfered with when the infra-red radiation was removed by means of a water filter. The removal of the ultra-violet radiation below 3000 A by means of an appropriate glass filter inhibited all activation. This definitely located the region in that part of the spectrum between 2000 and 3000 A.

Experiments with Monochromatic Radiation

The next step was to employ monochromatic radiation. We used for this purpose a technique similar to one described previously (Hollaender and Claus, 1936), with certain modifications. A diagram of the experimental arrangement is given in Fig. 1. *A* is the light source (water-cooled high pressure quartz capillary lamp, loc.) using the current from a 500 volt d.c. supply, burning on about 350 volts and 3.5 amperes.[1] *C* are quartz condensing lenses. *D* are quartz prisms mounted on an adjustable turn-table. *E* is a right angle quartz reflection prism mounted in such a manner that it can be turned through an

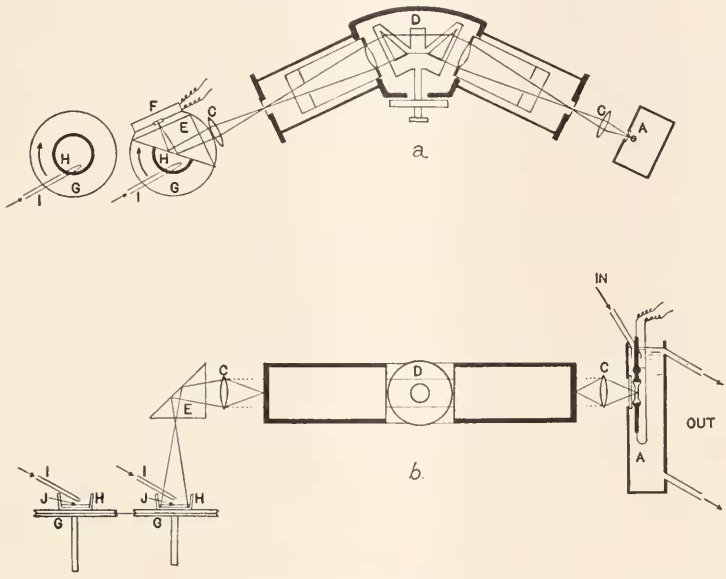

Fig. 1. Diagram of experimental arrangement for the exposure of *Arbacia* eggs with monochromatic radiation. *a*, horizontal, and *b*, vertical cross section. For details see text.

angle of 90°. This makes it possible for the reflected beam to cover a horizontal area of the exposure dish or the vertical receiving surface of the thermopile. *F* is a vacuum thermopile in casing with quartz window connected with a high sensitivity galvanometer (not shown in diagram). The sensitivity of the thermopile was determined against a Bureau of Standards "standard lamp." *G* are two platforms rotated by a small motor (not shown) at identical slow speed. *H* are small

[1] Special thanks should be given here to Mr. Boss and Dr. Pond of the Marine Biological Laboratory who made available a rectifier-transformer delivering the necessary high voltage.

(33 mm. diameter) Stender dishes each fastened to the center of a platform. *I* are the tapered outlets which direct a slight stream of air against the surfaces of the liquids in *H*.

For each experiment, the eggs of one *Arbacia* were washed, and about 300 to 600 eggs placed in each Stender dish, and covered to the height of about 3 mm. with sea water. The dish to be exposed was fastened to the platform under the reflection prism, the control dish was fastened to the platform to the left. The two platforms (*G*) with the dishes (*H*) were made to rotate at a uniform speed. Slight air streams of the same pressure for both dishes were directed against the outer quarters of the water surfaces. The rotation of each dish had a tendency to move the eggs against the outer rim, and the air stream tended to bring the eggs to the center of the dish. These two opposed forces made the eggs roll in the dish in such a manner that each egg occupied every possible position in the dish many times during the course of exposure, and every section of the surface of each egg received equal quantities of the radiation.

The efficiency of the stirring was checked in the following manner: Uranium glass particles were placed in the dish with sea water, the dish stirred as described above, and the beam of ultra-violet permitted to fall on the dish. The fluorescing moving glass particles illustrated the efficiency of stirring. If this method of stirring is used carefully, slow rotation, not too strong a beam of air, no damage will be done to the eggs. These precautions were necessary since the beam of monochromatic radiation covered only one-fifteenth of the bottom of the exposure dish. No other mechanical stirring device was found which would fulfill the condition outlined without damage to the eggs.

Before the actual exposure was started, the reflection prism was turned 90° so that the beam from the monochromator would fall on the receiving surface of the thermopile (*F*) as shown in the diagram of the horizontal cross section of the apparatus (*a*). After the energy reading was taken, the prism was turned through 90° and the beam made to cover the described area of the exposure dish as shown in the vertical cross section (*b*), having the dishes rotate during the entire time. Most exposures were for 3 to 10 minutes, but exposures as short as 30 seconds or as long as 30 minutes were made occasionally. Energy readings were taken before and after each exposure. If the exposure was longer than 15 minutes, an additional reading was taken during the middle of the exposure.

The effect of radiation of the following wave-lengths was tested: 2260, 2300, 2380, 2480, 2537, 2650, 2805, 2950, 3050, 3150, 3350, and 3650 A. Since activation was produced with whole eggs only by wave-lengths below 2805 A, and especially with radiation of 2260 to 2380,

most of the work described concerns itself with these latter wave-lengths.

After irradiation the eggs were removed to larger dishes and covered with more sea water. Usually the eggs were divided into several lots. One lot kept at room temperature for 4 to 5 hours and then 2 drops of 2 per cent formaldehyde per dish added, and the number of cleaved and uncleaved eggs determined. Another lot kept in the refrigerator at about 10° C. for 15 to 20 hours and then the percentage of cleaved eggs determined. It was necessary to keep the eggs at two different temperatures since the laboratory temperature during the summer of 1937 would rise often to 27°, a temperature critical for the development of the eggs. Apparently the cold seemed to be beneficial.

It is known that *Arbacia* eggs can be activated by many chemicals and by certain physical treatments beside radiation. Special care was taken to recognize such effects if they were produced inadvertently, in these experiments, and to avoid the error of interpreting them as radiation effects. For this reason each group of experiments had the following controls:

1. Eggs of each sea urchin used were fertilized with fresh sperm and only those eggs used which when tested gave a high percentage of cleavage.

2. Each group of four to five exposures had one standard control. That is, these control eggs were handled in an identical manner with the exposed eggs with the exception that they did not receive any ultra-violet radiation. Controls included in the lots were kept at room temperature as well as at 10° C. These controls gave a check in regard to mechanical as well as temperature effects.

3. In the course of this investigation, controls of the following type were used, one to each group of experiments. Dishes with the usual quantities of sea water but without eggs were irradiated with the different wave-lengths and given the different times of exposure found effective in the regular tests. After radiation, unfertilized eggs were added to the irradiated water and permitted to stand with the dishes of exposed eggs. With the wave-lengths and the times of exposures used in this investigation, no effect by irradiated sea water on the eggs could be recognized. If the time of exposure was extended for many hours, effects of the radiation on sea water were found (Rakestraw and Hollaender, 1936). But this lies outside the subject of this paper.

Results

The results of a typical experiment are given in Table I. The manner of calculating the incident energy/egg is illustrated in the following protocol.

TABLE I

Dish	Time of exposure	Incident energy/egg	Log of incident energy	Eggs cleaved : not cleaved	Percentage cleaved
83	30 seconds	0.335	0.525 −1	3 : 100	3
79	60 seconds	0.67	0.826 −1	68 : 220	23.8
77	120 seconds	1.37	0.137	107 : 226	32.1
75	240 seconds	2.67	0.426	283 : 73	79.5
73	360 seconds	4.0	0.602	316 : 62	83.5
82	0 (control)	0	0	0 : 252	0
76	240 seconds	2.67	0.426	268 : 31	89.5
78	120 seconds	1.37	0.137	241 : 81	75
79	0 (control)	0	0	0 : 212	0

Protocol of Part 2 of Experiment 28 August 8, 1937. Eggs removed at 10 A.M. washed twice with sea water, some fertilized, cleaved normally. Kept dish in sea water sink until eggs were needed. Standard exposure dish used. Exposed to 2260 A, 37.5 cm. galvanometer deflection, 61.3 ergs/cm. deflection. The beam covered $2 \times 28 = 56$ mm.^{2}, diameter of dish 33 mm., area $= .7854 \times 33^2 = 855$ mm^2. Part of area of the dish exposed $\frac{56}{855} = 15.3^{-1}$. Formula for calculation of incident energy received per egg with diameter of 74μ.

$$\frac{E \times T \times C \times f}{a} = I$$

E = Incident energy in ergs/second $= 37.5 \times 61.3$
T = Time of exposure = 30 seconds
C = Cross-sectional area of the egg 4300.8μ^2
a = Area of beam $= 56 \times 10^6 \mu^2$
f = Fraction of time each egg exposed $= \frac{1}{15.3}$
I = Incident energy/egg

$$\frac{37.5 \times 61.3 \times 30 \times 4300.8}{56 \times 10^6 \times 15.3} = 0.348 \text{ ergs/egg}$$

The eggs kept in the first six dishes as given in Table I were held at room temperature until 4 P.M. and then formaldehyde added. Duplicate set of dishes kept in refrigerator for 20 hours. Results of 3 dishes kept in refrigerator are given. Eggs in rest of dishes kept in refrigerator used for cytological examination.

Figure 2 illustrates the results of a typical experiment if one wavelength only was investigated, permitting the determination of many points on the curve. One curve is for eggs kept at room temperature

and the other curve for those kept at 10° C. It would appear that the eggs in the dishes kept at room temperatures were given the formaldehyde which stopped their development too early. This was unavoidable, since it was not always possible to determine in advance best time of cleavage, and waiting too long at room temperature brought about occasionally cytolysis of the eggs.

There was a considerable amount of variation in the eggs of different females in their sensitivity to radiation. Thus the incident energy of activation at wave-length 2260 A varied between 2.25 to 4.2 ergs per egg for 80 per cent activation. For this reason the activation energy at different wave-lengths was always determined for the eggs from one

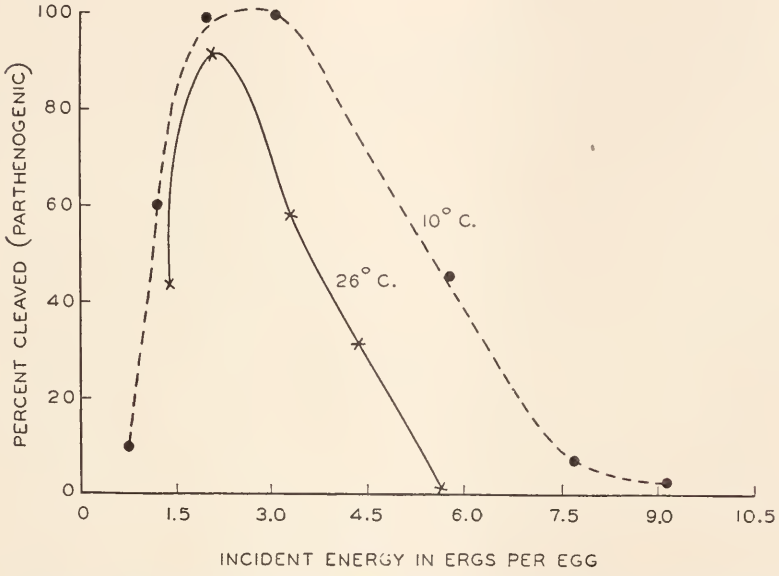

FIG. 2. Graphs showing the percentage of unfertilized eggs cleaved and the incident energy per egg. One set of eggs was kept at 26° C. and one at 10° C. after exposure.

individual and in no case has the relative energy necessary to produce activation in its dependence on wave-length been any different from that given in Fig. 3. That is, the lowest energy necessary to produce activation was at 2260 A and activation became negligible around 2500 A, even with relatively high energy values. Figure 3 is not as complete as is desirable because the number of exposures that can be handled in a certain time is limited. Complete curves as given in Fig. 2, if obtained for many wave-lengths, require more time than would be safe to keep eggs from one individual.

The irradiated eggs showed normal fertilization membranes. But

only eggs which showed at least the two-cell stages were called activated. This took usually three to five hours at room temperature. Many of the activated eggs went to further stages of development. Sixteen and 32-cell stages were observed, but some of the eggs cytolyzed after two or three divisions. It was necessary to limit this part of the investigation to the observation of divisions which gave the most reliable counts, i.e., the two-cell stage.

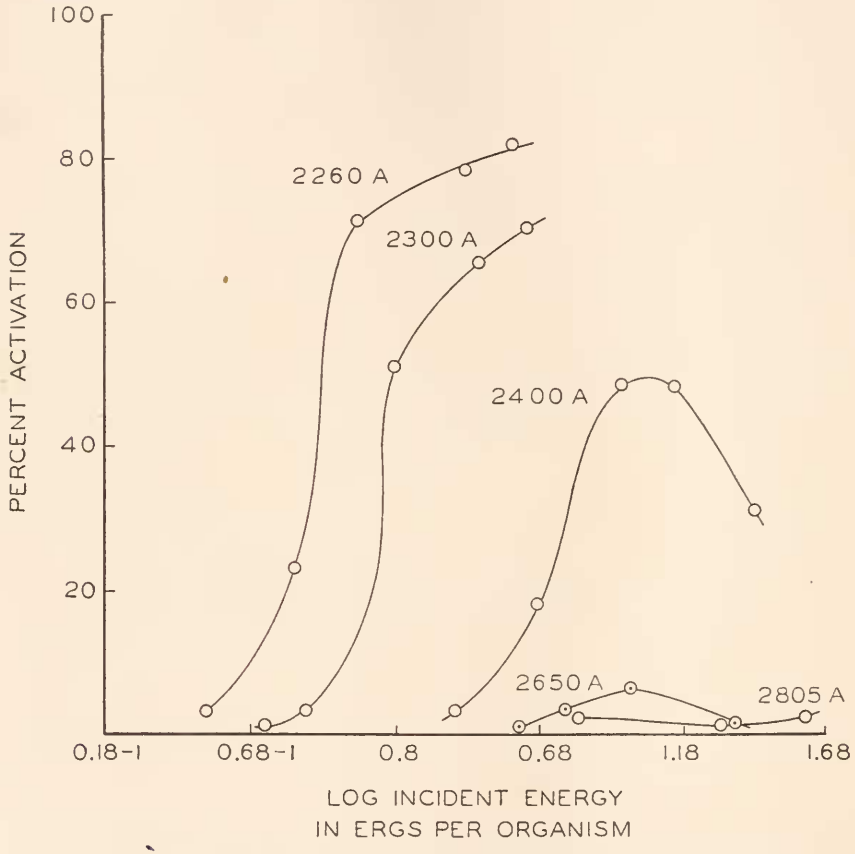

FIG. 3. Graphs showing percentage of the unfertilized eggs activated by monochromatic ultra-violet radiation against the log of the incident energy at five wave-lengths.

Figure 4 illustrates graphically the effectivity of the different wave-lengths in producing activation of *Arbacia* eggs, taking the energy necessary at 2260 A as 100. The absorption spectrum of an egg of *Paracentrotus lividus* as determined by Vlès and Gex (1927) is given. Also, the inactivation spectrum of the virus of typical tobacco mosaic

FIG. 4. Summary graph giving (1) the efficiency of the different wave-lengths in producing 50 per cent activation in *Arbacia* eggs; (2) The destruction curve of urease as described by Kubowitz and Haas (1933); (3) the destruction curve of the virus of typical tobacco mosaic (Hollaender and Duggar, 1936); and (4) the absorption curve of one egg of *Paracentrotus lividus* as given by Vlès and Gex (1928).

The data for the relative wave-length dependence of the action of monochromatic ultra-violet radiation on these different agents have been recalculated from the originals to make a comparison possible.

(Hollaender and Duggar, 1936) and the inactivation spectrum of urease (Kubowitz and Haas, 1933—see also Gates, 1934) are given. Data from these different sources have been recalculated to fit into the scheme of this graph and are given only for comparison, i.e., to show

the efficiency of different wave-lengths to bring about the investigated effects, and should not be compared for absolute energy values.

The absorption spectrum of an egg of *Paracentrotus lividus* as given by Vlès and Gex is not too helpful as a clue in interpreting the activation (parthenogenesis) spectrum, since it is that of a different species of sea urchin. The taking of such absorption spectrum is difficult and it is felt that it would be very worth while to repeat this work using a somewhat different technique from that used by Vlès and Gex. Coloring matter and other highly absorbing materials contained in the eggs, which vary for different species, mask probably to a large degree the absorption of the essential parts of the egg. However, the rapid increase of the absorption at wave-lengths between 2325 and 2300 (the lowest wave-length tested by Vlès and Gex) indicates a possible correlation with the activating radiation.

The inactivation spectra of urease and a typical virus have been given since these curves follow closely the activation curve of *Arbacia*. The virus curve has been suggested (Hollaender and Duggar, 1936) as the inactivation curve of a protein. The isolation of a crystalline protein with high virus activity from a typical tobacco mosaic virus suspension has been described by Stanley (1937). All these compounds, i.e., proteins, show their highest sensitivity in the ultra-violet below 2400 A. Nucleic acids have their most pronounced absorption around 2650 A (see also Caspersson, 1937) a wave-length not effective in producing activation of *Arbacia* eggs. It would appear from this that the possibility of the eggs being activated by a mechanism which starts with the absorption of the ultra-violet by nucleic acids is excluded. But not too definite conclusions as to the type of compound can be made from the high sensitivity of the *Arbacia* at the short ultra-violet, since besides proteins many other types of organic compounds also increase absorption at these short wave-lengths. We know very little of the effect of monochromatic ultra-violet radiation on proteins, especially the effect of energies which have been used in these tests.

Cytological observations in connection with this work are given in a separate paper (Harvey and Hollaender, 1938).

I wish to thank Dr. E. B. Harvey for her coöperation throughout the course of these experiments and for her criticism of this paper, the University of Wisconsin for placing laboratory space at the Marine Biological Laboratory at our disposal, and the Radiation Committee, National Research Council, for the supply of equipment.

Summary

1. A method for the effective irradiation of *Arbacia* eggs with measured quantities of monochromatic ultra-violet radiation is described.

2. The activation (parthenogenesis) of *Arbacia* eggs by polychromatic and monochromatic radiation is demonstrated.

3. The energy requirements and wave-length dependence of activation have been worked out. The wave-lengths most effective are in the shorter ultra-violet.

4. The wave-length dependence of the activation curve of *Arbacia* is compared with the inactivation curves for urease and the virus of typical tobacco mosaic.

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